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FINAL REPORT

ON

PERCUTANEOUS ABSORPTION

TO

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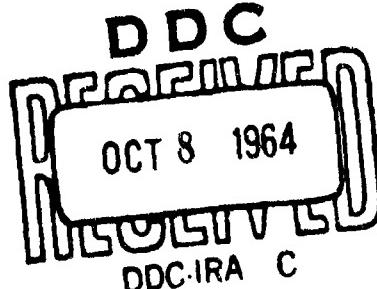
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This is a summary of work on percutaneous absorption carried out in our laboratories during the past five years. Reprints of 12 of our publications are included. In addition to these, there are 4 more papers which will be published within the next year. The bibliography lists these publications of our group under numbers 1-16. Also, much of this material is presented in previous progress reports (Progress Reports 1-6) from our group to the Army Chemical Center.

Our initial efforts were primarily concerned with a group of homologous compounds (nicotinic acid, methyl nicotinate, ethyl nicotinate, butyl nicotinate, hexyl nicotinate, octyl nicotinate and tetrahydrofurfuryl nicotinate, a related compound). These agents were applied to humans *in vivo*. Both topical applications and intradermal injections were on young adult, healthy volunteers. The biological activity (induction of erythema) was found to be in the range of 10^{-9} (minimal effective dose) or all these chemical agents when they were injected intradermally. However, when they were applied topically to human skin, the minimum effective dose for induction of erythema was quite different for the different compounds tested. For example, methyl nicotinate was effective in concentrations 133 times less than that of nicotinic acid. When one compared the ether/water partition coefficients of these compounds as well as absolute solubilities in water and ether, it seemed evident that these physical factors had some correlation with the ability of the nicotinic acid derivatives to penetrate the skin.

Table V, page 340 of reference #1 summarizes this information and suggests that agents with high solubility in both ether and water are likely to penetrate the skin better than agents which have a low solubility in one of these solvents even if it has a high solubility in the other solvent.

In this first series of *in vivo* experiments we did not cover the areas

of topical application of the nicotinic acid and its derivatives after they were applied to the skin. We were unaware of the volatility of some of these agents. Had we covered the sites and prevented evaporation, such volatile agents as ethyl and methyl nicotinate probably would have given erythema in much lower concentrations than those we reported.

The next field of study was that of studying penetration of C¹⁴ ethyl nicotinate and C¹⁴ nicotinic acid into human skin in vitro (2,3,4). Skin removed during surgical procedures was used for these studies. Mostly leg and breast skin was available. Six different hospitals in the Cleveland area cooperated with us in providing the fresh skin. Penetration chambers were designed so that skin could be draped over one open end and secured to the sides. The corium side was covered with saline. The epidermis was outside and the C¹⁴ agents were applied to the epidermis. The epidermis was covered with aluminum foil when ethyl nicotinate was used.

The most surprising observation was that C¹⁴ ethyl nicotinate penetrated human skin (in vitro) 37,000 times as fast as C¹⁴ nicotinic acid. If the epidermis was removed from the corium and the C¹⁴ agents applied to the corium, the penetration of both agents was essentially the same. Without the epidermis, the barrier to penetration of C¹⁴ nicotinic acid completely disappeared. This is best shown in Table I of reference #2 on page 1104.

If the epidermal surface was layered over with water before application of ethyl nicotinate, penetration was enhanced about six fold for this particular compound. This suggested that hydration facilitates penetration of the skin by chemical agents (3).

Simultaneous with the above work, we also tested the in vitro penetration of a group of phenylboronic compounds given to us by Dr. A.H. Solway. The

original interest of Solway was to test the ability of different phenylboronic compounds to penetrate the "blood-brain barrier". He chose compounds with different solubilities in water and lipid. He concluded that agents with a partition coefficient closer to one were the agents which penetrated best. Using human skin in vitro, we were able to demonstrate a twenty fold difference in penetration of human skin by this group of compounds. The phenylboronic compounds which penetrated best were those with an aqueous/benzene partition coefficient closer to one. Table I, page 48 of reference #5 summarizes this information.

Further work was undertaken to determine if regional differences exist in humans with regard to penetration of various agents (6). We used agents that would induce at visible biologic response when they reached the corium. These included (1) wheal reaction with histamine, (2) vasoconstriction with privine and (3) vasodilation with ethyl nicotinate.

The first obvious finding was that concentrated solutions of any of these agents did not penetrate the palms or soles. There were definite differences in penetration of the skin of different regions. In general, the agents penetrated the forehead, mid-chest and mid-back better than the arms or legs. There were differences among the agents tested. Regional differences up to eleven fold were found for one of the agents (privine). These results are summarized in Tables IV and V, pages 268-269 of reference #6.

In vivo estimation of effects of prehydration of skin on penetration of privine and ethyl nicotinate was done. The hydration was mild but even so differences of 6-12 fold were seen. Prehydration definitely increased penetration of the agents (Reference #6, page 269, Table VI).

A large number of volunteers (young adults) were used to assay the comparative vasoconstrictor abilities of a number of vasopressor agents. It was

found that vasoconstriction in skin is correlated with certain chemical structures and that many vasopressor agents were not vasoconstrictor agents in human skin (7). The agents which did give vasoconstriction in human skin by intradermal injection were assayed for ability to induce vasoconstriction by topical application. It was found, in general, that agents capable of inducing vasoconstriction intradermally (0.1 cc) at 10^{-6} or 10^{-7} required concentrations topically (0.1 cc) of 10-20% to induce constriction (see Progress report #3). This served to emphasize the highly effective barrier properties of the epidermis to this type of compound. If the horny layer was stripped off (Scotch tape method) concentrations of 10^{-6} would give vasoconstriction. Thus the barrier properties are contained somewhere in the horny layer, above the granular layer of human skin.

A follow up of the vasoconstrictor work led us to test the ability of an O-methyl transferase inhibitor (pyrogallol) to prolong the activity of norepinephrine injected into human skin (8). Evidence was found for O-methyl transferase in human skin.

Another physiologic method to test absorption was tried. Antimetabolites (5-fluoruracil, 6-mercaptopurine and aminopterin) were applied topically to the skin of rabbits to prevent hair growth. We were able to show that intradermal 5-fluoruracil would inhibit hair growth but that topical application of this agent would not prevent hair growth locally in rabbits. Simple topical application to human skin did not show any biologic effects but when applied under occlusive wrap, biologic effects were observed (see reference #9)

The next major area of interest was in exploring the effect of environment on percutaneous absorption. It was shown that occlusive wrapping of human skin with Saran wrap⁺ will cause intensive hydration of the horny layer and an increase

⁺ Saran wrap - Dow Chemical Company, Midland, Michigan

of surface temperature to 37°C., the same as internal body temperature (10, 11, 12).

Glucocorticosteroids were used because of their ability to induce vasoconstriction when applied topically or injected intradermally (10). Human volunteers were used for these studies. Various concentrations of a given steroid were applied to one forearm and covered with an occlusive wrap for 16 hours. To the opposite forearm the same concentrations of the same steroid were applied and the area covered with an aluminum guard which was perforated and bridged over the areas of application of the steroid. This was also left open and in place for 16 hours. When the two arms were compared, the arm covered with the occlusive film invariably showed vasoconstriction at a concentration 100 fold lower than the arm with the perforated guard. This was shown for over 19 different glucocorticosteroids tested in over 200 subjects (10, 11). Thus, the humid, hot environment of the occlusive film enhanced penetration by a factor of 100 over the ambient conditions (indoor-Winter-Cleveland, Ohio). This same series of experiments (11) also revealed that the phosphate salt of a glucocorticosteroid is much less active in human skin than its parent alcohol or the acetate when applied locally. The acetate derivative was more active than the parent alcohol in all cases (10, 11). Even more striking was the fact that triamcinolone is 1/10,000 as active topically on human skin as triamcinolone acetonide (Table I; reference 11, page 612). These remarkable differences in activity were surprising and remain unexplained.

Another observation of the effectiveness of occlusive films in enhancing penetration was that of inducing severe toxic reactions in a group of subjects when 0.02 ml. of 20% naphazoline base was applied to each forearm and covered with Saran (12). The toxic effects were noted in a few hours. The same amount of naphazoline applied topically without occlusive film gave no toxic effects whatever (6).

One of our most recent findings has been that of the keratin layer functioning as an effective reservoir for topically applied chemicals long after the chemical is applied to the surface of the skin (13, 14). This was first observed with glucocorticosteroids when 2-4 days after topical application vasoconstriction can be induced in the same area by occluding that area with Saran wrap. This effective reservoir has been shown to last for up to 17 days and for 7 days on the average in over 250 experiments with human volunteers. This reservoir has definitely been shown to exist to the horny layer (13, 14). Thus, any chemical agent applied to the horny layer has the potential and probability of staying there for long periods of time. This is a new concept in cutaneous pharmacophysiology and one that should have great importance in the field of cutaneous medicine as well as toxicology and pharmacology.

Early in 1963, we purchased a chamber which regulates humidity and temperature within a wide range and variation. This has been used to study in vitro penetration of C¹⁴ labelled chemicals through human skin. Considerable work has been done with C¹⁴ acetylsalicylic acid (15). Twenty fold increases are consistently seen when a temperature of 40°C. and humidity of 88% is compared with a temperature of 10°C. and humidity of 40%. Both temperature and humidity have independent effects on percutaneous absorption.

We have worked with an anticholinergic agent (AHR 483) in some detail to test its ability to penetrate human skin. This agent will inhibit sweating at concentrations of 10⁻³ intradermally (0.1 cc). It will inhibit sweating when applied topically in 0.1-0.5% concentration (0.1 cc. to 3cm.²). C¹⁴ AHR 483 was shown to penetrate human skin in vitro (37°C., humidity 88%) at a rate of 0.02% in 24 hours. This indicates a slow rate of penetration but the agent is effective in such low concentrations once it reaches the sweat gland that it is a potent pharmacologic agent by topical application (16).

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